Some authors have suggested that HCC patients may have mutated estrogen receptors that cannot be blocked by tamoxifen,[307] but by megestrol.[308] Again, the small number of patients in which this agent has been tested prevents any firm conclusion.

Transarterial Embolization and Chemoembolization

HCC exhibits intense neo-angiogenic activity during its progression.[150] At very early stages the tumor is not highly vascularised and its blood supply comes from the portal vein. As the tumor grows the blood supply becomes progressively arterialized, so that even well differentiated HCC is mostly dependent on the hepatic artery for blood supply. This characteristic provides the pathologic basis for the radiological characteristics that are used to diagnose the disease. It also provides the rationale to support arterial obstruction as an effective therapeutic option. Acute arterial obstruction induces ischemic tumor necrosis with a high rate of objective responses. Hepatic artery obstruction is performed during an angiographic procedure and is known as transarterial, or transcatheter arterial embolization (TAE). When TAE is combined with the prior injection into the hepatic artery of chemotherapeutic agents, usually mixed with lipiodol, the procedure is known as transarterial chemoembolization (TACE). Hepatic artery obstruction can be achieved by the injection or placement of several agents. Gelfoam carefully prepared as 1mm cubes is the most frequently used agent, but polyvinyl alcohol,[309] alcohol,[310] starch microspheres,[311] metallic coils[312] or even autologous blood clots[313] have also been used. Gelfoam powder should be not be used as this may cause biliary damage.[314]

The procedure requires the advancement of the catheter into the hepatic artery and then to lobar and segmental branches aiming to be as selective as possible so as to induce only minimal injury to the surrounding non-tumorous liver. Multifocal HCC involving both hepatic lobes may require the obstruction of the total hepatic artery blood flow.

Chemotherapy has to be injected prior to arterial obstruction. It is usual to suspend chemotherapy in lipiodol, an oily contrast agent used for lymphographic studies. Lipiodol is selectively retained within the tumor and this expands the exposure of the neoplastic cells to chemotherapy. The dose of chemotherapy to be administered has to be distributed among the affected lobes. If the tumor affects only one lobe, it is common policy to inject 25% of the agent into the lobe free of tumor with the objective of treating potentially undetected clones. Several chemotherapeutic agents have been used for TACE, but the most common is to inject adriamycin or cisplatin.[315]

TAE and TACE are considered for patients with non-surgical HCC that are also ineligible for percutaneous ablation, provided there is no extrahepatic tumor spread. The main contraindication is the lack of portal blood flow (because of portal vein thrombosis, portosystemic anastomoses or hepatofugal flow). Patients with lobar or segmental portal vein thrombosis are poor candidates for TACE, as this will cause necrosis of the tumor and of the non-tumorous liver deprived of blood supply. This increases the risk of treatment-related death due to liver failure. Patients with advanced liver disease (Child–Pugh class B or C) and/or clinical symptoms of end-stage cancer should not be considered for these treatments as they have an increased risk of liver failure and death.

The side effects of intra-arterial injection of chemotherapy are the same as for systemic administration: nausea, vomiting, bone marrow depression, alopecia and potentially renal failure. Hepatic artery obstruction with acute ischemia of the HCC is associated with the so-called post-embolization syndrome. This appears in more than 50% of the patients and consists of fever, abdominal pain and a moderate degree of ileus. Fasting is required for 24 hours and IV hydration is mandatory. Prophylactic antibiotics are not routinely used[316] Fever is a reflection of tumor necrosis, but a minority of patients may develop severe infectious complications such as hepatic abscess or cholecystitis. The post-embolization syndrome is usually self-limited in less than 48 hours and the patients can be discharged from the hospital.

Both TAE and TACE induce extensive tumor necrosis in more than 50% of the patients.[192] Treatment response is assessed by the decrease in the concentration of tumor markers and the identification of large intra-tumoral necrotic areas and reduction in tumor burden in dynamic CT or MRI.[3] Immediately after arterial obstruction it is possible to see intra-tumoral bubbles that reflect tissue liquefaction The evaluation of the treatment response should take into account the induction of intra-tumoral necrotic areas in estimating the decrease in tumor load, and not just a reduction in overall tumor size.[3] According to conventional WHO criteria the reported rate of objective responses ranges between 16% and 60%,[192,315] there being no differences between TAE and TACE. Fewer than 2% of treated patients achieve a complete response. During follow-up the residual tumor nests recover their blood supply and the tumor continues to grow. This consideration prompts treatment repetition either at regular intervals or "a la demande" as there is no prospective comparison to support one or other strategy.[315]

The tumor progression rate is reduced after treatment and this translates into a lower risk of vascular invasion.

Response to treatment is associated with a significant improvement in survival. Cumulative meta-analysis of all published RCT indicate that patient survival is significantly improved.[184] Until very recently, the gain in survival reported in individual trials was not statistically significant.[194,317-319] However, studies performed in Barcelona[320] and Hong Kong[321] reported a significant impact on survival have changed this negative statement. It has to be emphasized that the available trials are heterogeneous both in terms of patients profile, treatment schedule and agent used. Thus, it has still to be determined which are the best obstructing agents, the optimal chemotherapeutics and the most effective re-treatment schedule.

The improvement in survival in treated patients ranges from 20% to 60% at 2 years,[315] but it is clear that the relevance of the improvement as compared to their outcome if untreated, is largely dependent on the patients baseline characteristics regarding tumor stage, liver function and general health status.

Recommendations

19. TACE is recommended as first line non-curative therapy for non-surgical patients with large/multifocal HCC who do not have vascular invasion or extrahepatic spread (level I).

20. Tamoxifen, antiandrogens, octreotide or hepatic artery ligation/embolization are not recommended (level I). Other options such as radio-labelled Yttrium glass beads, radio-labelled lipiodol or immunotherapy cannot be recommended as standard therapy for advanced HCC outside clinical trials.

21. Systemic or selective intra-arterial chemotherapy is not recommended and should not be used as standard of care (level II).

Treatment Algorithm

As previously stated, the establishment of an evidence-based treatment strategy for HCC patients relies on fewer than one hundred RCT, assessing all of the possible treatment strategies. Almost all the treatment recommendations, therefore, are based on a critical reading of observational studies. In the clinical setting patients should be stratified by disease stage. For each stage there should be an indicated treatment. This is the basis for the BCLC scheme as depicted in Fig. 2.[17,19,322] The strategy combines in a single proposal staging, indicated treatment and estimation of prognosis, and it can be applied to the majority of patients evaluated for HCC.

Patients diagnosed at an early HCC stage are optimal candidates for resection, liver transplantation or percutaneous ablation. Resection is considered for patients with single tumors, absence of clinically relevant portal hypertension and normal bilirubin. Tumor size is not a limiting factor, but it is uncommon to resect patients with tumors >5cm. Transplantation is considered in patients with 3 nodules <3 cm or with single tumors ≤5 cm with liver function impairment precluding resection. If a long waiting time (>6 months) is expected resection or percutaneous treatments are recommended prior to OLT. Living donor transplantation should also be considered. Percutaneous ablation is indicated in patients with small non-surgical HCC. If these options are not feasible, patients have to be considered for palliation.

Transarterial chemoembolization is indicated in asymptomatic patients with multinodular tumors that have not invaded vessels nor been disseminated outside the liver. This type of patient is the best candidate for this approach, particularly if they still meet the criteria for Child–Pugh A stage. Treated patients who respond to therapy have an improved survival and thus, this is the last effective option in conventional clinical practice.

Patients who present with a more advanced stage because of liver failure or tumor growth with vascular involvement/extrahepatic spread or physical impairment reflected by a markedly impaired performance status (<2)[189-191] will not benefit from any treatment option, even one with known efficacy in earlier disease. Accordingly, the optimal policy for these subjects is to attempt to enroll them in research studies testing new agents either within phase 2 investigations or within RCT. The optimal design of such studies should be the comparison of any intervention vs. placebo or the best supportive care as currently practiced. There is no proof that any of the available agents has any impact on survival.

Finally, patients at a terminal stage with deeply impaired physical status (performance status >2) and/or massive tumor burden with heavily impaired liver function should receive symptomatic treatment to avoid unnecessary suffering.

Future Perspectives

This practice guideline has depicted the current status regarding the diagnosis, staging and treatment of HCC. As discussed, there are several areas where active research is needed, ranging from molecular pathogenesis to detection, diagnosis and treatment. The elucidation of the molecular steps that determine the transition from non-malignant to malignant should allow the stratification of patients according to the distinct pathways that led to cancer and also provide for new preventive and therapeutic strategies. Identification of new biomarkers to establish the risk of cancer and/or detect its appearance at a preclin-

ical stage are urgently needed. The current therapeutic approach also needs significant improvement. Treatments able to provide initial cure are hampered by a significant rate of disease recurrence and there is also a need for effective adjuvant therapies. Finally, the therapeutic options for patients with advanced HCC have limited impact and thus, development of new agents and strategies for this group of patients is of major relevance. Fortunately, the awareness of these needs by official agencies such as the National Institutes of Health has increased the resources allocated for sponsoring research in this area. Hence, the action plan of the liver disease section (www.niddk.nih.gov/fund/divisions/ddn/ldrb/ldrb_action_plan.htm) includes specific goals in the field of liver cancer. Hopefully, in the years to come the management of patients with HCC will offer a completely different perspective in which both prevention and treatment will have significantly decreased the number of HCC related deaths.

Acknowledgement: The authors thank the AASLD Practice Guidelines Committee for their help in the preparation of this practice guideline. This committee, in concert with F. Fred Poordad, M.D., and Margaret C. Shuhart, M.D., M.S., as the lead reviewers, provided extensive peer review of this manuscript. The members of the Practice Guidelines Committee include K. Rajender Reddy, M.D., Chair, Andres Cardenas, M.D., MMSc, Robert L. Carithers, Jr., M.D., Stanley M. Cohen, M.D., Timothy J. Davern, M.D., Thomas W. Faust, M.D., Steven L. Flamm, M.D., Gregory J. Gores, M.D., Steven-Huy B. Han, M.D., Elizabeth Hespenheide, MSN, ACNP, Michael R. Lucey, M.D., David R. Nelson, M.D., F. Fred Poordad, M.D., Margaret C. Shuhart, M.D., MS, Brent A. Tetri, M.D., Zobair M. Younossi, M.D., MPH, and Nizar N. Zein, M.D..

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HBsAg Seroclearance in Chronic Hepatitis B in the Chinese: Virological, Histological, and Clinical Aspects

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Few studies have examined Chinese patients with chronic hepatitis B who exhibit hepatitis B surface antigen (HBsAg) seroclearance. We comprehensively studied the biochemical, virological, histological, and clinical aspects of 92 patients with HBsAg seroclearance (median follow-up, 126 months). Ninety-two HBsAg-positive controls matched for age, sex, and duration of follow-up were also recruited. Liver biochemistry, serum hepatitis B virus (HBV) DNA levels, and development of clinical complications were monitored. Intrahepatic total and covalently closed circular (ccc) HBV DNA were measured quantitatively in 16 patients. HBV genotype was determined in 30 patients. The mean age at HBsAg seroclearance was 48.8 (+ 13.81) years. There was a significant improvement in serum alanine aminotransferase levels after HBsAg seroclearance ($p<0.0001$). Patients with genotype B had a higher chance of HBsAg seroclearance than those with genotype C ($P = .014$). Ninety-eight percent of patients had undetectable serum HBV DNA. Thirty-seven percent of patients had low titer of intrahepatic HBV DNA, mainly in the form of cccDNA (71%-100%). All 14 patients with liver biopsies had near normal histology. There was no difference in the risk of development of hepatocellular carcinoma (HCC) between patients with and without HBsAg seroclearance. However, the mean age of HBsAg seroclearance was significantly older in patients with HCC than in patients without HCC ($P = .016$). In conclusion, patients with HBsAg seroclearance had favorable biochemical, virological, and histological parameters. Intrahepatic HBV DNA level was low and predominantly in the form of cccDNA. However, HCC could still develop, particularly in patients with cirrhosis who had HBsAg seroclearance at an older age. (HEPATOLOGY 2004;39:1694–1701.)

Hepatitis B surface antigen (HBsAg) seroclearance is a rare event in Chinese patients with chronic hepatitis B virus (HBV) infection who acquire the disease early in life. The estimated annual incidence of HBsAg seroclearance is 0.1% to 0.8%.[1,2] Undetectable HBsAg in the serum is usually due to a decrease in viremia rather than the emergence of HBsAg mutants.[3] Some studies demonstrate a favorable outcome in terms of histological features and development of hepatocellular carcinoma (HCC).[2,4] However, these findings are not confirmed by other studies.[5,6]

The presence of HBV DNA within the liver in patients with HBsAg seroclearance has been demonstrated in some studies,[7–9] but it is not known whether the intrahepatic HBV DNA is replicative or nonreplicative—that is, in the form of covalently closed circular (ccc) DNA. Also, it is uncertain how much residual virus is in extrahepatic reservoirs like peripheral blood mononuclear cells (PBMC).

Therefore, we sought to determine the factors associated with and the significance of HBsAg seroclearance in Chinese HBV patients by examining the following parameters: (1) liver biochemistry, (2) HBV DNA in the serum and PBMC, (3) HBV genotypes, (4) intrahepatic total and cccDNA, (5) liver histology, and (6) the development of cirrhosis-related complications and HCC.

Abbreviations: HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cells; anti-HBs, antibody to HBsAg; HBeAg, hepatitis Be antigen; anti-HBe, antibody to HBeAg; PBS, phosphate-buffered saline.

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Received October 8, 2003; accepted March 5, 2004.

Supported by a research grant from the University of Hong Kong, Department of Medicine.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.20240

Table 1. Oligonucleotide Sequences for Each Invader* Assay Set

Assay	Oligonucleotide Type	Sequence (5′-3′)	Location (HBV Genome)
Set MS1	Invader* oligonucleotide	5′-ctcatctgccggwccgtgtgcacttcgt-3′	1565-1591
	Primary probe	5′-cgcgccgaggcttcacctctgcacgt-NH3-3′	1592-1606
	FRET cassette	5′-F-tctZagccggttttccggctgagacctcggcgcg-3′	
Set PS2	Invader* oligonucleotide	5′-gtckccatgcracgtgcagaggtgaat-3′	1618-1593
	Primary probe	5′-aggccacggacggcgaagtgcacacg-NH3-3′	1592-1579
	FRET cassette	5′-F-actZagccggttttccggctgagtcgtccgtggcct-3′	
hgDNA	Invader* oligonucleotide	5′-ccagcctccttagatcacagctccggaagt-3′	
hIGF gene	Primary probe	5′-atgacgtggcagaccagcactcatccacga-3′	
	FRET cassette	5′-F-tctZagccggttttccggctgagagtctgccacgtcat-3′	

Abbreviations: F, fluorophore; Z, quencher; hg, human genomic; hIGF, human insulin growth factor.
*Third Wave Technologies, Inc. Madison, WI.

Patients and Methods

Patients

Between August 1975 and October 2001, a total of 3,843 chronic HBV patients had been followed up every 3 to 6 months in the Hepatitis Clinic of the University of Hong Kong Queen Mary Hospital in Hong Kong. Liver biochemistry and HBV serology, including HBsAg, antibody to HBsAg (anti-HBs; for patients who became HBsAg-negative), hepatitis Be antigen (HBeAg), and antibody to HBeAg (anti-HBe) were checked (Abbott Laboratories, Chicago, IL). Ninety-two patients (HBeAg/anti-HBe, 21:71 on presentation) had HBsAg seroclearance. Six patients had prior interferon-alfa treatment. HBsAg seroclearance was defined as loss of serum HBsAg on repeated testing for a period of at least 6 months and during subsequent follow-up until the time of analysis. None of the patients had concomitant hepatitis C and hepatitis D infection. Patients with cirrhosis-related complications or HCC on presentation were excluded.

Ninety-two consecutive HBV patients presenting during the same period of time and matched for sex, age, and HBeAg/anti-HBe status on presentation and for the duration of follow-up were recruited as controls.

Measurement of HBV DNA Levels in Serum, PBMC, and Liver

Serum obtained at the last follow-up was tested for HBV DNA using the Cobas Amplicor HBV Monitor Test (Roche Diagnostics, Branchburg, NJ; lower limit of detection, 200 copies/mL). PBMC were isolated by standard Ficoll-Hypaque density gradient centrifugation from heparinized peripheral blood. The cell pellets were washed 3 times in phosphate-buffered saline (PBS) and resuspended in 200 μL of PBS. Total cellular DNA was isolated from the PBMC by the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), with a final elution volume of 200 μL. HBV DNA isolated from PBMC was measured by the Cobas Amplicor HBV Monitor Test. A TaqMan PCR Reagent Kit (Applied Biosystems, Foster City, CA), which contained specific primers and probe for detection of β-actin gene was used to measure the amount of human genomic DNA. The final concentrations of the primers and probe in each reaction were 300 μmol and 200 μmol, respectively. Real-time polymerase chain reaction (PCR) amplification and detection was performed with an ABI Prism 7000 Sequence Detection System (Applied Biosystems).

Liver biopsies were performed in 16 patients with HBsAg seroclearance (2 were subsequently found inadequate for histological assessment), 13 control patients, and 9 non-HBV subjects. Liver samples (0.5-1.5 cm in length) were homogenized in PBS with a pellet pestle-microtube set (Kontes Glass Co., Vineland, NJ), followed by DNA extraction with a QIAamp DNA Blood Mini Kit. The extracted DNA was eluted in 400 μL of the elution buffer.

The amount of total HBV DNA, cccDNA, and human genomic DNA were quantified using the Invader HBV DNA Assay (Third Wave Technologies, Inc., Madison, WI). The general principle of the Invader assay has been described.[10-12] Briefly, 2 oligonucleotides, the primary probe and Invader oligonucleotide, hybridized to a target DNA sequence to form a partially overlapping structure. A Cleavase enzyme recognized this structure and cut off a short nucleotide fragment (called a 5′-flap) from the primary probe. At an isothermal reaction temperature close to the melting temperature of the primary probe and lower than the melting temperature of the Invader oligonucleotide, the primary probe cycles on the target DNA, and the released 5′-flaps were amplified in proportion to the concentration of the target DNA. Fluorescence resonance energy transfer (FRET) cassettes were used to react with the released flaps and generate a fluorescence signal, which was measurable by real-time PCR machines.

Three sets of Invader assays were used; the sequences of the oligonucleotides for each of the Invader assay sets are shown in Table 1. Set MS1, designed for the detection of

the minus-strand sequences of the HBV genome, was used for quantitation of all replicative forms of HBV DNA. Set PS2, designed for the detection of plus-strand sequences with the cleavage site located at the 5′ base of the direct repeat (DR)-2 element, was used to determine cccDNA. A third set of Invader assay was designed for quantitation of the human insulin growth factor I (hIGF1) gene. Since the Cleavase acting site for set PS2 is at the 5′ base of the DR2 element, cleavage is specific for cccDNA, which contains a covalent linkage of the 3′ end of the plus strand to the 5′ base of the DR2 element.

Fifteen microlitres of heat-denatured DNA extracted from the liver biopsy samples was added to 5 μL of a reaction master mix containing 40 mmol/L 3-(N-Morpholino) propane sulfonic (pH 7.5), 50 mmol/L $MgCl_2$, 2 μmol/L primary probe, 2 μmol/L Invaderoligonucleotide, 1 μmol/L FRET cassette and 50 ng Cleavase. The reaction mixtures were then incubated in the Rotor-Gene 2000 Real-time Cycler (Corbett Research, Mortlake, Australia) with a temperature setting of 80°C for 2 minutes, followed by a single temperature incubation of 64°C for 240 minutes, with fluorescence signal reading at 1-minute intervals.

Signal generation of the Invader assay follows quadratic kinetics, and there is a linear relationship between the target copy concentration and the quadratic coefficient.[10] The amount of total HBV DNA and cccDNA in a sample was calculated by extrapolation from a standard curve of the quadratic coefficients generated by reactions using the probe sets MS1 and PS2 on external plasmid standards of known concentrations. Intrahepatic total HBV DNA and cccDNA levels were then standardized with respect to the amount (in nanograms) of human genomic DNA present in the samples, as determined by hIGFI detection by the Invader assay. Cell number was calculated based on the estimate of 6.667 pg of human genomic DNA per cell. Experiment with serial diluted plasmid standards shows that the Invader assay has a lower limit of detection of 0.002 HBV DNA copies/cell for both the relaxed circular and ccc forms of HBV DNA and a dynamic range of 5 orders of magnitude. Intrahepatic total HBV DNA and cccDNA levels were then standardized with respect to the amount (in nanograms) of human genomic DNA present in the samples, as determined by hIGFI detection by the Invader assay. The intrahepatic total HBV DNA measurement was verified by using Cobas Amplicor HBV Monitor Test and β-actin gene measurement mentioned above.

HBV Genotyping

Serum from 30 patients with HBsAg seroclearance (serum at the time of presentation before HBsAg seroclearance) and 50 controls were tested for HBV genotypes. Briefly, HBV DNA was extracted as described in Stuyver et al.[13] The HBsAg region spanning aa107-205 was amplified and analyzed by INNO-LiPA HBV Genotyping (Innogenetics NV, Ghent, Belgium). The INNO-LiPA HBV Genotyping assay contains oligonucleotide probes specific for HBV genotypes A to G applied as 14 different lines on a membrane strip. Multiple probes are present for each HBV genotype. The correct HBV genotype was determined by consulting an interpretation chart showing probe reactivity patterns for each genotype.

Histological Assessment

Histological activity index scores were assessed according to the criteria of Knodell et al.[14] Immunoperoxidase staining was performed using monoclonal antibodies against HBsAg and HBcAg (Signet, Dedham, MA; dilution, 1:2).

Statistical Analysis

All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 10.0 for Windows; SPSS Inc., Chicago, IL). All continuous variables were tested for normality by Kolmogorov-Smirnov test. Comparison between continuous variables with normal distribution was tested by Student t test. Continuous skewed variables were tested by Mann-Whitney U test and Kruskal-Wallis test for 2- and 3-group comparison, respectively. Two related skewed variables were tested by Wilcoxon signed rank test. Correlation between 2 variables was tested by Spearman rank correlation. Categorical variables were tested by chi-square test or Fisher exact test.

Results

Demographic Data and Liver Biochemistry on Presentation

Demographic data for the 92 patients with HBsAg seroclearance and 92 control patients are listed in Table 2. There were no significant differences in any of the parameters between the 2 groups.

HBeAg Seroconversion and HBsAg Seroclearance. The 21 HBeAg-positive patients with subsequent HBsAg seroclearance had HBeAg seroconversion at a mean (± SD) age of 38.09 (+ 16.65) years. The median alanine aminotransferase (ALT) decreased from 99.5 U/L (range, 13-3990 U/L) to 22 U/L (range, 9-1806 U/L; $P < .0001$) after HBeAg seroconversion. Only 14 of the 21 HBeAg-positive patients in the control group had HBeAg seroconversion.

The mean age of HBsAg seroclearance for the 92 patients was 48.8 (+ 13.81) years. Fifty-four patients

Table 2. Demographic Data and Liver Biochemistry on Presentation for 92 Patients With HBsAg Seroclearance and 92 Control Patients

	Patients with HBsAg Seroclearance	Control (Patients without HBsAg Seroclearance)
Demographic data		
Number	92	92
M/F	65:27	65:27
Mean age (±SD)	42.55 (14.37)	41.90 (15.09)
HBeAg/anti-HBe	21:71	21:71
Median duration of follow-up, mo (range)	126.28 (32.3-282)	125.50 (42-315)
Median duration of follow-up after HBsAg seroclearance, mo (range)	51.12 (18.72-199.56)	–
Liver biochemistry		
Median albumin, U/L (range)	47 (26-56)	47 (24-58)
Median bilirubin, μmol/L (range)	11 (3-553)	10 (2-76)
Median ALT, U/L (range)	27 (6-3390)	31 (8-1020)
Median AST, U/L (range)	24 (10-614)	30.5 (13-476)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

(58.7%) had detectable anti-HBs with the median level of 60.8 mIU/mL (range, 4.4-1000 mIU/mL). With HBsAg seroclearance, the median ALT of the 92 patients improved from 22 U/L (range, 6-1806 U/L) to 19 U/L (range, 6-106 U/L; $P < .0001$).

There was no significant difference in liver biochemistry on presentation between patients with HBsAg seroclearance and controls (Table 2). The control patients had a lower incidence of HBeAg seroconversion though they were of similar age and had similar duration of follow-up (p = .009). For the control who had HBeAg seroconversion, the median peak ALT levels during HBeAg seroconversion was similar to that of patients with subsequent HBsAg seroclearance: 89 U/L (range, 6-819 U/L) versus 136 U/L (range, 8-1806 U/L), respectively ($P = .4$). The distribution of HBV genotypes is listed in Table 3. Patients with HBsAg seroclearance were more likely to be of genotype B ($P = .014$).

HBV DNA in Serum and PBMC. Only 1 patient (1.1%) had a detectable HBV DNA level of 1870 copies/mL at a median interval of 49.1 months (range, 10-116.77 months) after HBsAg seroclearance. Sixty-six patients had their PBMC isolated for the determination of HBV DNA levels at a median interval of 48.8 months (range, 18.7-126.8 months) after HBsAg seroclearance. None had detectable HBV DNA in the PBMC, though human genomic DNA was detected by real-time PCR for β-actin.

Table 3. HBV Genotype Distribution of Patients With and Without HBsAg Seroclearance

No. of Patients	Patients With HBsAg Seroclearance (n = 30)	Patients Without HBsAg Seroclearance (n = 50)
Single genotype*		
A (%)	1 (3.3)	2 (4)
B (%)	16 (53.3)	15 (30)
C (%)	10 (33.3)	32 (64)
D (%)	2 (6.7)	–
Mixed genotypes		
A + D (%)	1 (3.3)	–
B + C (%)	–	1 (2)

*Among patients with single genotype B or C, those with HBsAg seroclearance had a higher proportion of genotype B and lower proportion of genotype C when compared to controls; odds ratio 3.41; (95% CI, 1.26-9.28); $P = .014$.

Intrahepatic Total HBV DNA and cccDNA. Results of the intrahepatic HBV DNA are listed in Table 4. For the 16 patients with HBsAg seroclearance, the median interval between the time of HBsAg seroclearance and the time of liver biopsy was 47.9 months (range, 26.4-127.9 months). Six patients (37.5%) had detectable intrahepatic total HBV DNA levels, 4 of whom were positive for anti-HBs in the serum. There was a significant decreasing trend in median intrahepatic total HBV DNA levels (expressed in copies per nanogram of human genomic DNA) for HBeAg-positive patients (17613.26 copies/ng [range, 370.14-32956.14 copies/ng]); anti-HBe–positive patients (2229.0 copies/ng [range, 25.19-13347.66 copies/ng]); and patients with HBsAg seroclearance (undetectable level [range, undetectable level-12.02 copies/ng]); $P < .0001$. There was good correlation between the intrahepatic total HBV DNA levels measured by the Invader assay and Cobas Amplicor HBV Monitor Test ($r = 0.92$, $P < .0001$).

Intrahepatic HBV DNA in the 6 patients with HBsAg seroclearance and measurable intrahepatic HBV DNA was predominantly in the form of cccDNA, whereas most of the intrahepatic HBV DNA of the 13 control patients had relatively low percentages of cccDNA: median percentage of cccDNA, 100% (range 71.28%-100%) versus 5.82% (range, 2.56%-57.96%), respectively; $P < .0001$.

Liver Histology. The mean age of HBsAg seroclearance for the 14 patients with assessable liver biopsy was 41.96 months (+ 8.34). The median interval between the time of HBsAg seroclearance and liver biopsies was

Table 4. Intrahepatic Total HBV DNA and cccDNA of Patients and Controls

		Total HBV DNA		cccDNA by Invader Assay	
	Patients	Invader Assay	Cobas Amplicor HBV Monitor Test	cccDNA Level	cccDNA (%)
HBsAg seroclearance	1	12.02 (0.080)	2.51	12.02 (0.080)	100
	2	0.95 (0.006)	0.26	0.95 (0.006)	100
	3	0.96 (0.006)	0.12	0.87 (0.006)	91.07
	4	0.41 (0.003)	Undetectable	0.41 (0.003)	100
	5	2.51 (0.017)	Undetectable	2.52 (0.017)	100
	6	0.85 (0.006)	Undetectable	0.61 (0.004)	71.28
	7	Undetectable	0.48	Undetectable	NA
	8	Undetectable	0.22	Undetectable	NA
	9-16	Undetectable	Undetectable	Undetectable	NA
HBeAg + ve	17	32956.14 (219.72)	951.81	1118.9 (7.46)	3.4
	18	26544.14 (176.97)	1386.21	1381.63 (9.21)	5.21
	19	17613.26 (117.43)	8991.13	949.69 (6.33)	5.39
	20	7477.43 (49.85)	149.37	479.43 (3.20)	6.4
	21	370.14 (2.47)	56.95	60.99 (0.41)	16.48
Anti-HBe + ve	22	13347.66 (88.99)	368.68	536.82 (3.58)	2.56
	23	4527.04 (30.18)	277.57	263.68 (1.76)	5.82
	24	3656.05 (24.37)	107.13	105.26 (0.70)	2.88
	25	2848.33 (18.99)	93.76	142.42 (0.95)	5
	26	1609.66 (10.73)	185.79	107.63 (0.72)	6.69
	27	449.38 (3.00)	40.22	42.74 (0.28)	9.51
	28	74.44 (0.50)	3.31	29.56 (0.20)	39.71
	29	25.69 (0.17)	5.09	14.89 (0.10)	57.96
Non-HBV controls (n = 9)		Undetectable	Undetectable	Undetectable	NA

NOTE: HBV DNA and cccDNA expressed in copies per nanogram of human genomic DNA (copies per cell).
Abbreviation: NA, not applicable.

45.77 months (range, 26.4-66.02 months). The histological features of the liver biopsies are listed in Table 5. Of 14 patients, 12 (85.7%) had nearly normal liver histology. The remaining 2 had mild nonspecific changes. Abnormal histological activity index score was observed in all 4 patients who had HBsAg seroclearance after age 50 and in only 3 of 10 (30%) patients who had HBsAg seroclearance before age 50 ($P = .07$).

Cirrhosis-Related Complications and HCC One patient had esophageal varices after HBeAg seroconversion but before HBsAg seroclearance. Four patients had HCC developing at 20, 21.1, 48, and 65.2 months after HBsAg seroclearance. One patient had ascites at

Table 5. Histological Features of the 13 Patients With HBsAg Seroclearance

			Histologic Activity Index*				
Patients	Age of HBsAg Seroclearance, y	Interval Between Liver Biopsy and HBsAg Seroclearance, mo	Periportal Bridging Necrosis	Interlobular Degeneration and Focal Necrosis	Portal Inflammation	Fibrosis	Total Score
1	31.31	41.43	0	1	1	0	2
2	31.95	66.03	0	1	0	0	1
3	34.92	47.97	0	0	0	0	0
4†	36.61	33.47	0	0	0	0	0
5	36.95	127.9	0	0	0	0	0
6	38.72	26.4	0	0	0	0	0
7	39.17	47.8	0	0	0	0	0
8	39.46	29.23	0	0	0	0	0
9	39.74	38.9	0	0	0	1	1
10	45.27	56.37	0	0	0	0	0
11	51.64	29.6	0	0	0	1	1
12	51.88	49.5	0	0	1	0	1
13	51.92	65.57	0	1	1	0	2
14	57.94	43.73	0	1	0	0	1

*Histological activity index score: 0-10 for periportal bridging necrosis and 0-4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.
†Patient was positive for HBsAg by immunoperoxidase staining HBcAg immunoperoxidase staining was negative in all other patients.

57 months after HBeAg seroconversion and HCC at 9 months after HBsAg seroclearance. All 6 patients had undetectable HBV DNA in the serum. Of the 5 patients with HCC, all except one had biochemical and radiological evidence of cirrhosis. One patient with HCC had previous interferon-alfa treatment. There were 11 controls (1 positive for HBeAg, 10 positive for anti-HBe) who developed cirrhosis-related complications and/or HCC. Of these, 7 had HCC only, 1 had esophageal varices, ascites, and HCC, and 3 had ascites and/or esophageal varices.

There was no significant difference in the risk of HCC between patients with and without HBsAg seroclearance: 5 of 92 (5.4%) versus 8 of 92 (8.70%), respectively; $P = .39$]. However, the mean age of HBsAg seroclearance of the 5 patients who subsequently developed HCC was significantly older than that of the remaining 87 patients: 63.16 years (+ 8.27) versus 47.94 years (+ 13.64), respectively; $P = .016$.

Discussion

To our knowledge, the current study is the largest series studying the significance and various virological aspects of HBV patients with HBsAg seroclearance, though intrahepatic HBV DNA, liver histology, and HBV genotypes were determined in only a limited number of patients. Liver biochemistry on presentation and peak ALT levels during HBeAg seroconversion were of no predictive value for subsequent HBsAg seroclearance. Although 6 of 92 patients had prior interferon-alfa treatment, we have previously shown in a long-term follow-up study that interferon-alfa treatment does not significantly enhance HBsAg seroclearance in Chinese HBV patients.[15] However, earlier HBeAg seroconversion and genotype B (Table 3) were associated with a higher chance of HBsAg seroclearance. Further studies on the effect of genotype in HBV diseases are indicated because some studies have reported that genotype C is associated with more serious HBV disease.[16,17] It may also be worthwhile to study serum HBV DNA levels and/or HBsAg titers after HBeAg seroconversion to see whether they are of predictive value for future HBsAg seroclearance. The majority of our patients (>98%) had undetectable serum HBV DNA levels using a sensitive PCR assay at a median duration of 49 months after HBsAg seroclearance. This confirmed the findings of several studies that serum HBV DNA becomes undetectable 1 to 2 years after HBsAg seroclearance.[8,9,18–20] The extrahepatic reservoir for HBV DNA was also low since all 66 patients with PBMC testing were negative for HBV DNA. Mason et al. reported that 5 out of 12 patients with HBsAg seroclearance had HBV DNA in PBMC detectable by PCR.[21] The difference in the results between our study and Mason et al. might be due to the fact that 9 of the 12 patients studied by Mason et al. had HBV DNA measured within 24 months of HBsAg seroclearance, whereas in our study, the median interval between the measurement of HBV DNA in PBMC and HBsAg seroclearance was 48 months (only 3 of 66 patients had HBV DNA measurement within 24 months of HBsAg seroclearance). It is possible that the viral load in the extrahepatic reservoir decreases with time.

However, a substantial number of these patients (37.5%) still had detectable HBV DNA in the liver, a finding similar to other studies.[7–9,22] We demonstrated quantitatively, that there was a decreasing trend in the amount of total intrahepatic HBV DNA for HBeAg-positive patients, anti-HBe-positive patients, and patients with HBsAg seroclearance ($P < .0001$). We applied and verified a novel means to quantify the amount of intrahepatic HBV DNA as copies per nanogram of human genomic DNA and copies per cell in order to avoid the error introduced by a gross estimation of tissue weight with HBV DNA expressed as copies per unit weight of tissue.

The present study also showed that, in patients with measurable intrahepatic HBV DNA, it was mainly in the form of cccDNA after HBsAg seroclearance (Table 4). Though we were using the novel Invader assay to measure cccDNA, this assay was stringently verified by synthetic oligonucleotide target specificity assay, S1 nuclease treatment, and intra-assay and interassay variation experiments (data not shown). It has also been verified by PCR technique. cccDNA forms the template for the transcription of viral RNAs. It has been suggested that HBV inside the liver in patients with HBsAg seroclearance is probably in a complete and uninterrupted form.[8,9] The failure to detect mRNA in the liver by hybridization *in situ* also suggests that viral transcription is minimal or at a rate slower than that of cell turnover.[7] But in these previous reports, it was impossible to characterize the HBV DNA in the liver because the investigators were unable to detect HBV DNA by Southern-blot hybridization.[7,8] Our study suggests that the viral HBV DNA was predominantly in the form of cccDNA. However, the role of the integrated HBV DNA is not determined in the present study. The probes of the Invader assay used in this study would probably not detect integrated HBV DNA. Integration often occurs at the 11-base pair DR1 and DR2 regions, leading to breaching of the 2 regions,[23,24] and no hybridization would take place. Nevertheless, the presence of cccDNA in patients with HBsAg seroclearance may have some im-

plications for the potential future development of HCC in patients with HBsAg seroclearance (see below). However, in the majority of these patients, the viral load inside the liver should continue to decrease if the hepatocyte turnover rate exceeds the transcription rate of viral RNAs from the small amount of cccDNA. In theory, therefore, the HBV inside the liver should eventually disappear through natural cell death, though considerable time may be required even after HBsAg seroclearance.

Despite these favorable parameters in patients with HBsAg seroclearance, we were unable to show a significant decrease in the risk for the occurrence of HCC. This finding is similar to other studies.[5,6,22,25–28] Of the 5 patients who developed HCC after HBsAg seroclearance, 4 already had evidence of cirrhosis at the time of development of HCC. In addition, the HBsAg seroclearance of these 5 patients occurred at a relatively older age compared to the remaining 87 patients (mean 63.16 years vs. 47.94 years, respectively; $P = .016$). The longest interval between HBsAg seroclearance and the detection of HCC was 65.2 months. It is possible that cirrhosis of the liver, and probably the process of hepatocarcinogenesis (*i.e.*, the oncogenic effect of the integration of HBV DNA into the host), had already developed at the time of HBsAg seroclearance for these 5 patients. It is also possible that any residual cccDNA in the hepatocytes may still have had integrative capacity. Further studies are required to determine this. HBsAg seroclearance at a younger age is probably associated with a reduced risk of development of HCC. This is supported by the near normal histology of the 14 patients with assessable histology in whom the mean age of HBsAg seroclearance was 42 years. Further longitudinal follow-up of these patients for the occurrence of HBV-related complications is required to prove that HBsAg seroclearance at a relative young age confers good prognosis.

In conclusion, chronic HBV patients with HBsAg seroclearance are usually associated with favorable biochemical, virological, and histological parameters. Intrahepatic HBV DNA levels were extremely low and predominantly in the form of cccDNA. However, development of HCC is still possible, particularly in patients with cirrhosis who have HBsAg seroclearance at an older age.

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Lamivudine for Patients with Chronic Hepatitis B and Advanced Liver Disease

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ABSTRACT

BACKGROUND

The effectiveness of antiviral therapy in preventing disease progression in patients with chronic hepatitis B and advanced fibrosis or cirrhosis is unknown.

METHODS

Patients with chronic hepatitis B who had histologically confirmed cirrhosis or advanced fibrosis were randomly assigned in a 2:1 ratio to receive lamivudine (100 mg per day) or placebo for a maximum of five years. Of 651 patients, 436 were assigned to receive lamivudine and 215 to receive placebo. The primary end point was time to disease progression, defined by hepatic decompensation, hepatocellular carcinoma, spontaneous bacterial peritonitis, bleeding gastroesophageal varices, or death related to liver disease. An independent data and safety monitoring board monitored the progress of the study and performed interim analyses of the data.

RESULTS

We randomly assigned 651 patients (98 percent Asian and 85 percent male) to receive lamivudine or placebo. The study was terminated after a median duration of treatment of 32.4 months (range, 0 to 42) owing to a significant difference between treatment groups in the number of end points reached. End points were reached by 7.8 percent of the patients receiving lamivudine and 17.7 percent of those receiving placebo (hazard ratio for disease progression, 0.45; P=0.001). The Child–Pugh score increased in 3.4 percent of the patients receiving lamivudine and 8.8 percent of those receiving placebo (hazard ratio, 0.45; P=0.02), whereas hepatocellular carcinoma occurred in 3.9 percent of those in the lamivudine group and 7.4 percent of those in the placebo group (hazard ratio, 0.49; P=0.047). Genotypic resistance YMDD mutations developed in 49 percent of the patients treated with lamivudine, and the Child–Pugh score was more likely to increase in patients with these mutations than in the other patients treated with lamivudine (7 percent vs. <1 percent). Overall, 12 percent of the patients in the lamivudine group and 18 percent of the patients in the placebo group reported serious adverse events.

CONCLUSIONS

Continuous treatment with lamivudine delays clinical progression in patients with chronic hepatitis B and advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and the risk of hepatocellular carcinoma.

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N Engl J Med 2004;351:1521-31.

CHRONIC HEPATITIS B IS A SERIOUS problem worldwide.[1] Among patients with active viral replication, cirrhosis will develop in 15 to 20 percent within five years.[2,3] For patients with cirrhosis, acute exacerbation can occur, the disease may progress, and the incidence of hepatocellular carcinoma is greatly increased (70 to 90 percent of cases of hepatocellular carcinoma occur against a background of cirrhosis).[4,5] Because of these complications, five-year survival rates may be as low as 55 percent.[6] Ultimately, 40 percent of Asian men with chronic hepatitis B die of either complications of cirrhosis or hepatocellular carcinoma.[7]

Patients with persistent seropositivity for hepatitis B e antigen (HBeAg) or an increased serum alanine aminotransferase level after HBeAg seroconversion have a significantly increased risk of cirrhosis and hepatocellular carcinoma.[8-11] This is consistent with experimental models showing important roles for continuing hepatitis B virus (HBV) replication and the resultant hepatic inflammatory response in hepatocarcinogenesis.[12] Thus, the suppression of HBV and the reduction of necroinflammatory activity in chronic hepatitis B may prevent cirrhosis and, consequently, liver failure and hepatocellular carcinoma.[13]

Patients who have a response to interferon therapy have substantially fewer life-threatening liver complications than those who do not have a response,[14] although the evidence of the effect of this therapy on the incidence of hepatocellular carcinoma is less conclusive.[15-17] Use of interferon is restricted by cost, side effects, and, among patients with cirrhosis, the risk of liver failure during a flare of hepatitis. These limitations do not apply to oral antiviral agents, such as lamivudine, which can produce marked viral suppression, reduction of hepatic necroinflammatory activity,[18,19] histologic improvement of liver fibrosis,[20,21] and improved liver function,[22] even in patients with decompensation.[23,24] However, long-term therapy with lamivudine leads to viral breakthrough in some patients, owing to the emergence of genotypic resistance tyrosine, methionine, aspartate, aspartate (YMDD) mutations.[25] The possible implications of a resumption of necroinflammatory activity[21,26] include flares of hepatitis, which may lead to liver failure and death, and a gradual erosion of hepatic function, which may lead to decompensation or cirrhosis.

It has not been possible to devise treatment guidelines for the subgroup of patients with HBV-related cirrhosis or advanced hepatic fibrosis.[27,28] Therefore, we conducted a prospective, randomized, double-blind, placebo-controlled trial to assess the efficacy of lamivudine in terms of the clinical progression of disease in patients with chronic hepatitis B and advanced fibrosis or cirrhosis. This study was conducted at multiple centers in countries in the Asian–Pacific region, where chronic hepatitis B is a major cause of morbidity and mortality from cirrhosis and where hepatocellular carcinoma is a major cause of death.

METHODS

STUDY DESIGN

We planned to conduct this multicenter, centrally randomized, double-blind, placebo-controlled, parallel group study for five years or less. Patients were randomly assigned in a 2:1 ratio to receive lamivudine (100 mg per day) or placebo within 30 days after screening. Of 651 patients, 436 were assigned to receive lamivudine and 215 to receive placebo. During the double-blind phase, treatment was stopped for patients who reached a clinically confirmed end point (disease progression) or had HBeAg seroconversion. Patients who reached an end point were offered open-label lamivudine for one year, and patients who had HBeAg seroconversion were followed up after therapy and had the option to receive lamivudine as an open-label treatment in the event of serologic relapse. If the trial was terminated according to predefined criteria, patients were to be offered open-label treatment for one year.

The data reported in this article are from the double-blind phase of the study, including follow-up after treatment, up to the time of termination.

PATIENTS

Patients over 16 years of age with chronic hepatitis B were eligible for recruitment if they had been positive for hepatitis B surface antigen (HBsAg) for at least six months, were positive for HBeAg or negative for HBeAg with detectable HBV DNA at screening, and had had a liver biopsy showing an Ishak fibrosis score of at least 4 (where 0 indicates no fibrosis and 6 indicates cirrhosis) at screening or during the previous two years. Biopsy slides were reviewed by one centrally appointed independent

histopathologist who was blinded to the treatment assignments.

Patients were excluded if they had any of the following: evidence of hepatocellular carcinoma (suspicious foci on hepatic ultrasonography at screening or a rising serum level of alpha-fetoprotein), a serum alanine aminotransferase level more than 10 times the upper limit of normal, any evidence of hepatic decompensation (as defined by the study protocol), autoimmune hepatitis, coinfection with hepatitis C or D virus or human immunodeficiency virus, other serious concurrent illness (e.g., alcoholism, uncontrolled diabetes, or cancer), pancreatic amylase or lipase levels more than two times the upper limit of normal, an elevated serum creatinine level, a hemoglobin level of less than 8 g per deciliter, a white-cell count below 1500 per cubic millimeter, a platelet count of 50,000 per cubic millimeter or less, treatment with immunomodulatory or chronic antiviral therapy within the 6 months before screening, treatment with any investigational drug within the 30 days before the study began, or any previous treatment with lamivudine. Women who were pregnant were also excluded.

ASSESSMENTS

Patients were assessed at baseline, at the end of months 1 and 3, and at every three months thereafter for clinical evidence of hepatic decompensation or other complications. They were also questioned about adverse events, concurrent medications, and study drug accountability; blood was taken for hematology and biochemistry profiles; serum samples were tested for HBeAg, hepatitis B e antibody, and alpha-fetoprotein; and the prothrombin time was measured. At baseline and every six months thereafter, serum was assayed for HBsAg and hepatitis B surface antibody, and liver ultrasonography was performed. HBeAg seroconversion was considered confirmed if two consecutive samples taken at least a month apart were positive for hepatitis B e antibody and negative for HBeAg. Hepatic ultrasonography and liver biopsy or fine-needle aspiration were performed as clinically indicated to investigate or confirm a diagnosis of hepatocellular carcinoma.

Serum samples were collected at baseline and at months 1, 12, 24, 36, 48, and 60 and analyzed for HBV DNA levels at a central laboratory. HBV DNA was determined by a branched-chain hybridization assay (Versant HBV DNA Quantitative Assay, Bayer Diagnostics, with a lower limit of detection of 0.7 mEq per milliliter). Results were unavailable to the investigators until after the completion of the study, and serum HBV DNA assays were not permitted at the investigators' sites during double-blind therapy but were allowed after confirmed HBeAg seroconversion or during open-label lamivudine therapy. Samples collected at baseline, at annual visits, and at the completion of treatment were also analyzed for the presence of YMDD mutations by polymerase-chain-reaction assay and restriction-fragment–length polymorphism assay. Samples collected at all scheduled visits from patients with clinical end points were also tested for YMDD mutations.

END POINTS

The primary end point was time to disease progression, as defined by the first occurrence of any of the following: an increase of at least 2 points in the Child–Pugh score (an assessment of the severity of liver disease [range, 5 to 15, where 5 indicates good liver function and 15 indicates poor liver function] calculated on the basis of the serum bilirubin and albumin levels, the prothrombin time, and the presence and degree of ascites or encephalopathy), spontaneous bacterial peritonitis with proven sepsis, renal insufficiency, bleeding gastric or esophageal varices, the development of hepatocellular carcinoma, or death related to liver disease. Patients with a first clinical end point were followed for subsequent end points. Any increase in the Child–Pugh score due solely to laboratory parameters was confirmed on two consecutive visits at least one month apart. For patients with albumin levels below 35 g per liter or bilirubin levels greater than 34.2 μmol per liter (2 mg per deciliter) at baseline, confirmatory tests were conducted one week after the first test. Renal insufficiency was defined as a decrease in creatinine clearance to 50 ml per minute (0.8 ml per second) or less that was confirmed two times, at least one week apart. Hepatocellular carcinoma was diagnosed on the basis of results of ultrasonography, selective arteriography, imaging of hepatic tumors during the vascular phase, serum levels of alpha-fetoprotein, or by cytologic or histologic evaluation. The evidence for each end point was reviewed and confirmed by a blinded clinical end-points committee composed of three internationally recognized hepatologists.

Table 1. **Baseline Characteristics of the Patients.***

Characteristic	Lamivudine Group (N=436)	Placebo Group (N=215)
Male sex — no. (%)	370 (85)	182 (85)
Asian — no. (%)	426 (98)	210 (98)
Age — yr		
Median	43	44
Range	17–74	22–71
Child–Pugh score — no. (%)†		
5	341 (78)	156 (73)
6	75 (17)	41 (19)
≥7	20 (5)	18 (8)
Ishak fibrosis score — no. (%)‡		
4	176 (40)	76 (35)‡
5	127 (29)	55 (26)
6	133 (31)	84 (39)
HBV DNA — mEq/ml		
Median	11.7	21.5
Range	<0.7–109,800	<0.7–4234
HBV DNA ≥0.7 mEq/ml — no. (%)§	345 (79)	174 (81)
Positive for HBeAg — no. (%)	252 (58)	124 (58)
Alpha-fetoprotein — μg/liter		
Median	8.6	9.8
Range	0.7–600	1.2–298
Albumin — g/liter		
Median	42	41
Range	28–54	27–52
Alanine aminotransferase — U/liter		
Median	70	68
Range	14–959	7–821
Alanine aminotransferase >1 time the upper limit of normal — no. (%)	338 (78)	171 (80)

SAFETY

All adverse events, regardless of their possible association with the disease or study treatment, were recorded. Adverse events were considered to be serious if the investigator determined that they jeopardized the patient, were life-threatening, or would result in hospitalization, disability, or death.

DATA AND SAFETY MONITORING BOARD

The data and safety monitoring board consisted of three independent hepatologists, who were not members of the end-points committee, and an independent statistician. The board protected the ethical interests and safety of the patients by reviewing interim analyses. The board was empowered to recommend termination of the study on the basis of safety concerns or as soon as sufficient evidence indicated that lamivudine was statistically superior to placebo or that lamivudine did not provide a significant advantage over placebo.

STATISTICAL ANALYSES

Sample size was determined on the basis of the primary analysis of time to disease progression. To estimate power, the annual rate of disease progression was assumed to be 20 percent for the placebo group,[8,9,29] whereas a reduction in this rate of one third (to 13.3 percent) for the lamivudine group

Table 1. (Continued.)

Characteristic	Lamivudine Group (N=436)	Placebo Group (N=215)
Aspartate aminotransferase — U/liter		
Median	52	54
Range	14–686	17–367
Bilirubin — μmol/liter¶		
Median	13.7	13.7
Range	3.0–50.0	1.7–58.1
Creatinine — μmol/liter‖		
Median	88	88
Range	35–173	35–135
Hemoglobin — g/dl		
Median	14.7	14.6
Range	8.4–19.0	9.2–17.8
Platelet count per mm³		
Median	145,000	131,000
Range	14,000–401,000	41,000–360,000
Prothrombin time — sec		
Median	12.5	12.8
Range	8.0–23.8	9.8–27.6
White-cell count per mm³		
Median	5330	5300
Range	1980–11,600	2200–11,500

* There were no significant differences between the two treatment groups. The patients' race was recorded by the investigators, on the basis of the interviews and evaluations of the patients.
† The Child–Pugh score (range, 5 to 15, where 5 indicates good liver function and 15 poor liver function) is a measure of the severity of liver disease.
‡ The Ishak fibrosis score (range, 0 to 6) is a measure of the degree of fibrosis in liver-biopsy specimens. Scores of 0 to 4 indicate no or moderate fibrosis, and 5 or 6 severe fibrosis or cirrhosis.
§ All patients had detectable HBV DNA at screening; 0.7 meq per milliliter equals approximately 7×10^5 copies per milliliter.
¶ To convert values for bilirubin to milligrams per deciliter, divide by 17.1.
‖ To convert values for creatinine to milligrams per deciliter, divide by 88.4.

was considered to be a clinically relevant treatment effect. This difference corresponds to a hazard ratio of 0.64. For the study to have a power of 90 percent at the 5 percent level of significance, with a ratio of 2:1 for the random assignment of patients to lamivudine or placebo, 240 end points would need to be observed.[30] Assuming a dropout rate of 25 percent during a five-year period, the number of patients required overall was estimated to be 600.

We used a sequential, asymmetric trial with the triangular test[31] to monitor the primary efficacy end point of time to clinical disease progression. At each interim analysis, the test statistics were calculated and compared with straight-line stopping boundaries. At each inspection, the "Christmas tree" correction[31] was applied to the continuous boundaries to account for the unpredictable number and timing of interim analyses.

The first interim analysis was scheduled for 18 months after the completion of patient recruitment, and subsequent interim analyses were to be performed between 6 and 12 months after the first interim analysis; the aim was to have approximately 35 events between interim analyses. The intention-to-treat analysis included all patients who were randomly assigned to receive either lamivudine or placebo. Treatments were compared with the use of a Cox proportional-hazards model,[32] with each analysis allowing for the covariates of country, sex,

baseline alanine aminotransferase levels, and baseline Child–Pugh and fibrosis scores. The data from patients without end points were censored as of the date that treatment was stopped (if the data and safety monitoring board terminated the trial) or at the last date of available follow-up after treatment (if the trial was terminated for other reasons). Because the study was stopped at the second interim analysis with strict stopping criteria applied at the first interim analysis, adjustments that had to be made to the final P values and estimates were negligible (an increase in the P value of <0.001 and an increase of <0.002 for the hazard ratio).

The study was conducted in accordance with good clinical practice and all applicable regulations, including the Declaration of Helsinki (modified in 1996). Each investigator ensured that the protocol was reviewed and approved by the local ethics committee. Written informed consent was obtained from each patient before enrollment in the study.

The study was designed by the academic investigators in conjunction with medical staff from GlaxoSmithKline. The data were collected by the investigators and analyzed by GlaxoSmithKline. Each author had access to the data. This article was written by a committee consisting of seven authors (Drs. Liaw, Sung, Chow, and Farrell; and Mrs. Shue, Mr. Keene, and Dr. Dixon, who are GlaxoSmithKline employees). The committee members vouch for the validity and completeness of the data and the veracity of the data analysis.

RESULTS

CHARACTERISTICS OF THE PATIENTS

The intention-to-treat population consisted of 651 patients who were randomly assigned to treatment at 41 sites across Australia, China, Hong Kong, Malaysia, New Zealand, the Philippines, Singapore, Taiwan, and Thailand; 436 patients were assigned to receive lamivudine and 215 to receive placebo. In each treatment group, 85 percent of the patients were male and 98 percent were Asian. The treatment groups were also well matched in terms of age, laboratory results at baseline, and Ishak fibrosis scores (Table 1). The median Child–Pugh score at baseline was 5 (range, 5 to 9), and no patient had evidence of hepatocellular carcinoma, renal insufficiency, bleeding varices, or spontaneous bacterial peritonitis at study entry.

STUDY TERMINATION AND END POINTS

At the recommendation of the data and safety monitoring board, the double-blind phase of the study was terminated at the second interim analysis, because results had crossed the predefined boundary for showing efficacy. At this time, 67 patients had achieved HBeAg seroconversion, 52 had stopped therapy for other reasons, and 68 end

Table 2. Disease Progression during Double-Blind Treatment and Follow-up after Treatment.*

Variable	Lamivudine Group (N=436)	Placebo Group (N=215)	Hazard Ratio (95% CI)†	P Value
	no. of patients (%)			
Overall disease progression	34 (7.8)‡	38 (17.7)	0.45 (0.28–0.73)	0.001
Increase in Child–Pugh score	15 (3.4)	19 (8.8)	0.45 (0.22–0.90)	0.02
Hepatocellular carcinoma§	17 (3.9)	16 (7.4)	0.49 (0.25–0.99)	0.047
Renal insufficiency	2 (0.5)	0	—	—
Bleeding varices	2 (0.5)	3 (1.4)	—	—
Spontaneous bacterial peritonitis	0	0	—	—
Liver-related death	0	0	—	—

* Only one patient reached an end point during follow-up before the termination of the study. Dashes denote not applicable.

† Hazard ratios were derived from a Cox model adjusted for country, sex, baseline alanine aminotransferase level, Child–Pugh score, and Ishak fibrosis score. CI denotes confidence interval, unadjusted for interim analyses.

‡ Two patients fulfilled two criteria simultaneously at end-point confirmation.

§ When five cases of hepatocellular carcinoma diagnosed during the first year were excluded, the hazard ratio was 0.47 (95 percent confidence interval, 0.22 to 1.00; P=0.052).

points had occurred. Four additional end points occurred between the data cutoff for the second interim analysis and termination of the study, a period of 20 weeks. The median duration of treatment was 32.4 months (range, 0 to 42 months); 71 percent of the patients had received study medication for at least 30 months when the study was terminated.

Overall, 72 patients reached clinical end points; 34 of 436 (7.8 percent) in the lamivudine group and 38 of 215 (17.7 percent) in the placebo group (P=0.001) (Table 2). An increase in the Child–Pugh score occurred in 15 patients (3.4 percent) in the lamivudine group and 19 patients (8.8 percent) in the placebo group (P=0.02). Hepatocellular carcinoma occurred in 17 patients (3.9 percent) who received lamivudine and 16 patients (7.4 percent) who received placebo (P=0.047). There were no cases of death related to liver disease or spontaneous bacterial peritonitis that were not already accounted for by the other defined clinical end points, and only two cases of renal insufficiency and five cases of bleeding varices.

Kaplan–Meier estimates of the proportion of patients with disease progression after three years are shown in Figure 1. Hepatocellular carcinoma developed in five patients during the first year of the study, two in the placebo group and three in the lamivudine group. Even if these tumors had existed but had not been detected before study entry, the exclusion of the patients would not have affected the result of the primary analysis of time to disease progression. However, for the time to a diagnosis of hepatocellular carcinoma, the hazard ratio changed from 0.49 (P=0.047) to 0.47 (P=0.052).

The incidence of disease progression in various subgroups is shown in Table 3. Covariate modeling of time to disease progression showed that the factors other than treatment that significantly affected outcome were the Child–Pugh score at baseline and the Ishak fibrosis score at baseline. In both instances, higher scores were associated with a greater frequency of end points.

YMDD MUTATIONS

Two patients had evidence of YMDD mutations at baseline, and 5 patients had no samples after baseline, so data on the emergence of YMDD mutations during therapy were available for 644 patients. After baseline, at least one sample with evidence of YMDD mutations was found in 209 of 430 pa-

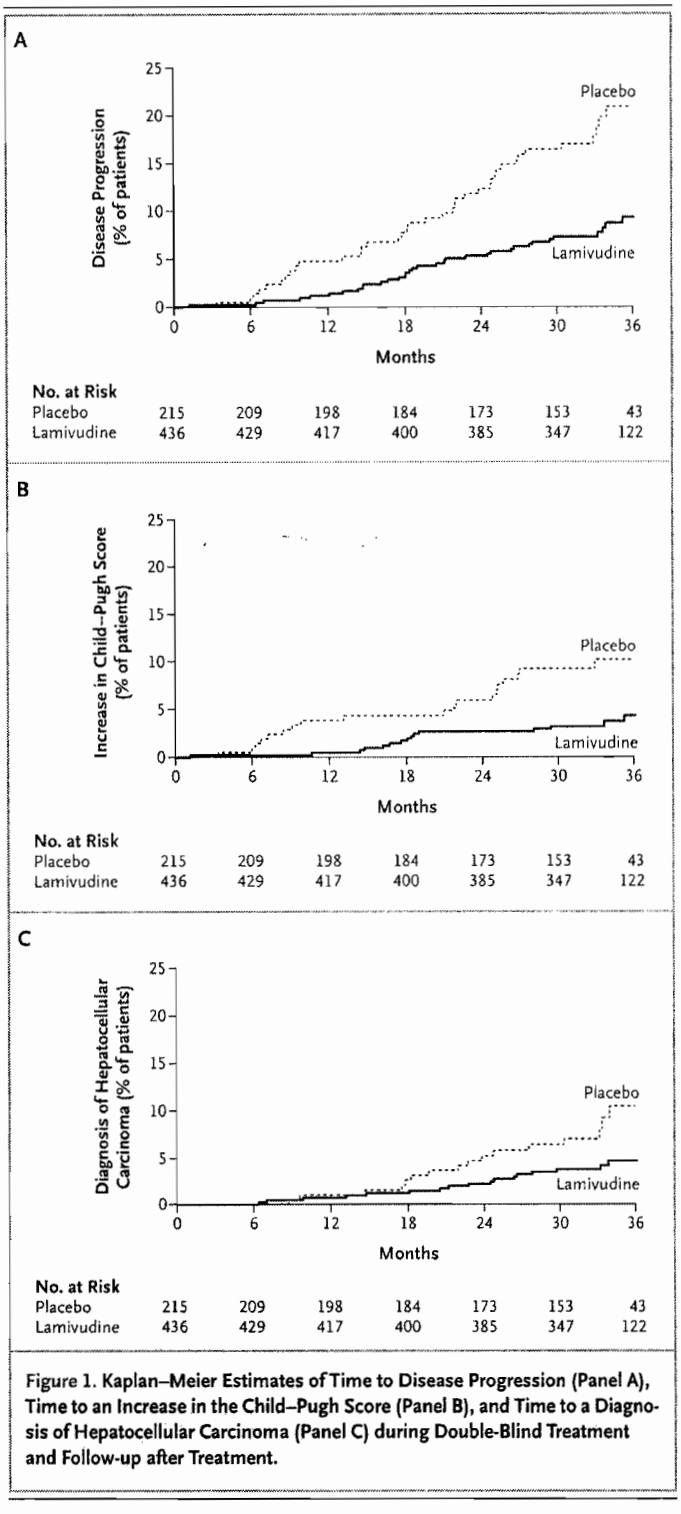

Figure 1. Kaplan–Meier Estimates of Time to Disease Progression (Panel A), Time to an Increase in the Child–Pugh Score (Panel B), and Time to a Diagnosis of Hepatocellular Carcinoma (Panel C) during Double-Blind Treatment and Follow-up after Treatment.

Table 3. **Association between Pretreatment Variables and the Incidence of Disease Progression.**

Variable at Baseline	Incidence of Disease Progression	
	Lamivudine Group (N=436)	Placebo Group (N=215)
	no./total no. (%)	
Sex		
Male	32/370 (9)	30/182 (16)
Female	2/66 (3)	8/33 (24)
Child–Pugh score		
5	16/341 (5)	18/156 (12)
6	9/75 (12)	12/41 (29)
≥7	9/20 (45)	8/18 (44)
Ishak fibrosis score		
≤4	8/176 (5)	9/76 (12)
5	11/127 (9)	9/55 (16)
6	15/133 (11)	20/84 (24)
HBeAg status		
Positive	15/252 (6)	25/124 (20)
Negative	19/182 (10)	13/91 (14)
HBV DNA		
Below the lower limit of quantitation	4/89 (4)	9/41 (22)
0.7–10 meq/ml	14/123 (11)	9/51 (18)
>10–100 meq/ml	6/103 (6)	8/50 (16)
>100 meq/ml	10/119 (8)	12/73 (16)
Serum alanine aminotransferase		
≤2 times the upper limit of normal	27/274 (10)	25/132 (19)
>2 times the upper limit of normal	7/162 (4)	13/83 (16)

tients (49 percent) in the lamivudine group and 11 of 214 patients (5 percent) in the placebo group. Only 5 percent of patients without YMDD mutations had detectable HBV DNA breakthrough, as compared with 62 percent of patients with YMDD mutations in the lamivudine group.

Patients in the lamivudine group who had YMDD mutations were more likely to have an increased Child–Pugh score than those without YMDD mutations ($P<0.001$), but they were less likely to reach an end point than were patients in the placebo group ($P>0.05$) (Table 4).

ADVERSE EVENTS

Overall, 12 percent of the patients in the lamivudine group and 18 percent of the patients in the placebo group reported serious adverse events. The incidence and nature of adverse events were similar among patients who received lamivudine and those who received placebo (Table 5). In addition, elevations in serum alanine aminotransferase to levels at least three times as great as the levels at baseline occurred in 12 percent of patients receiving lamivudine and 25 percent of patients receiving placebo.

There were 12 deaths among patients originally assigned to receive lamivudine and 4 among those originally assigned to the placebo group. Nine patients died while they were receiving lamivudine (seven during open-label treatment with lamivudine), and seven died during follow-up after treatment. Two patients in the lamivudine group died during double-blind therapy (1 died from preexisting lymphoma; the other drowned after a myocardial infarction), and 14 died after a clinical end point had been reached. These 14 deaths were attributed to hepatocellular carcinoma (8 patients) and an increased Child–Pugh score (6 patients). Eight of the 10 patients originally assigned to receive lamivudine who died after reaching a clinical end point had evidence of YMDD mutations.

DISCUSSION

The most important finding of this study is that lamivudine reduces the risk of liver complications for patients with chronic hepatitis B and cirrhosis or advanced fibrosis. The magnitude of protection conferred by lamivudine is substantial, with a reduction of approximately 50 percent in disease progression during a median period of 32 months of treatment.

The study was stopped early because the large and significant difference between the treatment groups with respect to the primary end point (time to disease progression) met the predefined efficacy criteria for termination. Treatment differences for individual end points were a secondary consideration, but the results showed significant differences between the two treatments with respect to both an increase in the Child–Pugh score and the incidence of hepatocellular carcinoma. Studies with longer follow-up and more potent or sustained therapy would be required to establish the full potential of antiviral therapy as a strategy to prevent liver cancer, to measure the potential improvements in survival, and to identify the subgroups of patients who would obtain the greatest benefit from treatment.

Chronic hepatitis B is a highly variable disease in which factors such as the age of the patient, the duration of infection, the immune response of the host, and the viral genotype influence the activity, rate of progression, and severity of liver disease.

Although our study was not powered for reliable subgroup analysis, pretreatment variables related to disease progression were high fibrosis scores and Child–Pugh scores at baseline. This is consistent with the high rates of hepatocellular carcinoma observed among patients with advanced stages of liver disease.[33]

The main reservation about the long-term use of lamivudine has been the emergence of YMDD mutations, which has occasionally been associated with severe, and even fatal, flares of hepatitis.[25,34] In light of this uncertainty, the finding that treatment with lamivudine for a median period of 32 months reduces the rates of hepatic decompensation and hepatocellular carcinoma without increasing the number of serious adverse events is important. Even among patients who developed YMDD mutations, clinical end points occurred less frequently than among patients receiving placebo. However, patients with YMDD mutations were more likely to have an increase in the Child–Pugh score and to die for reasons related to clinical end points than were those patients who did not have YMDD mutations. This may be because the resumption of viral replication restores the potential for facilitating disease progression. The long-term effects of lamivudine on disease progression are not known. Since the present trial was started, treatment with a combination of adefovir dipivoxil and lamivudine has been shown to suppress replication of YMDD mutations and improve liver function in patients with hepatic decompensation.[35] The adverse effects of YMDD mutations may be overcome by the addition of adefovir dipivoxil, but we did not assess this possibility in our population. The potential adverse effects of lamivudine treatment must be considered in any therapeutic plan.

In summary, this multicenter, prospective, randomized, double-blind, placebo-controlled trial of lamivudine in patients with chronic hepatitis B and cirrhosis or advanced fibrosis showed that lamivudine decreased progression of the disease, thereby reducing clinically important complications. In particular, treatment with lamivudine approximately halved the rate of hepatic decompensation during 32 months of continuous treatment and appeared to have similar efficacy in reducing the rate of hepatocellular carcinoma. The emergence of YMDD mutations reduced the benefit of lamivudine but did not negate it, despite the occurrence of more end points due to decompensation among patients with YMDD mutations than among those without the mutations. Our results provide the opportunity to develop strategies to achieve even better outcomes for patients with chronic hepatitis B and cirrhosis or advanced fibrosis by means of sustained viral suppression by minimizing or preventing the effects of drug resistance.

Table 4. Incidence of Clinical End Points According to Evidence of YMDD Mutations.

Clinical End Point	Lamivudine Group		Placebo Group (N=214)
	Negative for YMDD Mutations (N=221)	Positive for YMDD Mutations (N=209)	
	number (percent)		
Total	11 (5)	23 (11)	38 (18)
Increase in Child–Pugh score ≥2	1 (<1)	14 (7)	19 (9)
Hepatocellular carcinoma	8 (4)	9 (4)	16 (7)

Table 5. Incidence of Adverse Events during Double-Blind Phase.

Variable	Lamivudine Group (N=436)	Placebo Group (N=215)	P Value*
	no. (%)		
Death	2 (<1)	0	0.89
Any serious adverse event	54 (12)	38 (18)	0.09
Any adverse event†	335(77)	178 (83)	0.11
Ear, nose, or throat infections	97 (22)	44 (20)	0.67
Abdominal discomfort or pain	77 (18)	43 (20)	0.54
Malaise or fatigue	65 (15)	42 (20)	0.17
Headache	64 (15)	21 (10)	0.10
Cough	62 (14)	15 (7)	0.008
Diarrhea	33 (8)	29 (13)	0.03
Viral respiratory infections	39 (9)	21 (10)	0.84

* P values were calculated on the basis of the two-sided Fisher's exact test.
† The adverse events shown are those that occurred in at least 10 percent of the patients in a treatment group.

Supported by GlaxoSmithKline, Greenford, United Kingdom.

Dr. Liaw reports having received grant support from Gilead Sciences and Roche and serving on the International HBV Advisory Board of Roche. Drs. Sung and Farrell report having served as paid speakers for GlaxoSmithKline. Dr. Farrell reports having received consulting fees from Roche, Novartis, and Chiron. Drs. Dixon, Gray, and Sabbat and Mrs. Shue and Mr. Keene are employees of GlaxoSmithKline and, except for Mrs. Shue, have each reported equity ownership in GlaxoSmithKline.

We are indebted to A. Burroughs, G. Foster, R. Guan, and S. Todd of the Data Safety Monitoring Board; G.C. Farrell, C.L. Lai, and Y.F. Liaw of the Clinical Endpoint Committee; S.N. Huang, central histopathologist at Sun-Yat-Sen Cancer Centre, Taiwan; M. Rubin, P. Hodsman, and N. Brown at GlaxoSmithKline, and J. Whitehead and G. Nicholls at the University of Reading, United Kingdom, for their contributions to the design of the study; and to M. Sullivan, S. Garrido-Lestache, and H.H. Lew of GlaxoSmithKline for their assistance in the conduct of the study.

APPENDIX

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